RESEARCH NOTE

Molecular Identification of G6PD Chatham (G1003A) in Khuzestan Province of Iran

MARYAM GHADERI GANDOMANI, SAEID REZA KHATAMI*, SEYED REZA KAZEMI NEZHAD, SETAREH DANESHMAND and AMIR MASHAYEKHI

Department of Genetics, Faculty of Science, Shahid Chamran University of Ahvaz 65355141, Ahvaz, Iran

[Gandomani M. G., Khatami S. R., Nezhad S. R. K., Daneshmand S. and Mashayekhi A. 2011 Molecular identification of G6PD Chatham (G1003A) in Kheezestan Province of Iran. *J. Genet*. **90**, 143–145]

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is the first enzyme in pentose phosphate pathway and the main intracellular source of NADPH. Since G6PD is the only source of NADPH in red blood cells, defense against oxidative damage strongly depends on its activity (Mehta *et al.* 2000). Deficiency of G6PD enzyme in the red blood cells, under certain circumstances, may lead to an abnormal rupture of the cell wall with resultant hemolytic anemia (Glader and Lukens 1999). According to G6PD activity levels, WHO has classified different G6PD variants into five classes (WHO 1967). The *g6pd* gene is located on the Xq28 region and contains 13 exons which is approximately 18.5 kb in length (Beutler 1994). Glucose-6-phosphate dehydrogenase deficiency (G6PDD) is the most common X-linked recessive erythroenzymopathy among humans, estimated to affect more than 400 million people world wide (Mehta *et al.* 2000).

After the Mediterranean variant of G6PD, the Chatham mutation is the most common variant in several Middle-East countries and in some provinces of Iran (Rahimi *et al.* 2006). Substitution of adenine for guanine at nucleotide 1003 leads to substitution of alanine by threonine at amino acid position 335 (Vulliamy *et al.* 1988). G6PD Chatham was first reported in an Indian boy with neonatal jaundice, living in London, and subsequently was detected in several populations (Samilchuk *et al.* 2003). The highest frequency was reported from northern Iran (27%) (Mesbah-Namin *et al.* 2002) and the lowest frequency from Brazil (0.66%) (Saad *et al.* 1997). G6PD Chatham (Ala335Thr) is caused by G1003A mutation in exon 9 of *g6pd* gene. This mutation causes class II of G6PD deficiency characterized by possessing less than 10% of normal enzyme activity, which makes it one of the most severe forms of G6PD deficiency (Rahimi *et al.* 2006). In this note, we report a study of G6PD Chatham in Khuzestan Province and this is the first report based on the molecular analysis of G6PD Chatham in southwestern Iran.

Materials and methods

We performed DNA analysis on 231 G6PD deficient individuals. While 79 G6PD deficient individuals were selected from random screening of 1065 male blood donors for G6PD deficiency by fluorescent spot test (Beutler *et al.* 1979), a further 152 G6PD deficient patients (116 males and 36 females) were identified with favism or neonatal jaundice history hospitalized in different hospitals of Khuzestan Province, Iran. Samples were collected in 0.5 M EDTA falcon tubes and carried on ice to the laboratory and stored at −70°C until further use. Genomic DNA was extracted from whole blood of the G6PD deficient subjects using DNA extraction kit (Roche Molecular Biochemicals, Switzerland). All samples were analysed by PCR-RFLP method for the G1003A mutation in exon 9 which is characteristic of G6PD Chatham. For PCR reaction, the forward and reverse primers were: 5′-CAA GGA GCC CAT TCT CTC CCT T-3′ and 5′-TTC TCC ACA TAG AGG ACG ACG GCT GCC AAA GT-3′ respectively (Rahimi *et al.* 2006). The PCR amplification was carried out under the following conditions: 10 cycles of 94°C for 30 s and 66°C for 1 min, 72°C for 1 min and 20 cycles of 94°C, 65°C, 72°C each temperature for 1 min. Chatham PCR products were digested using restriction enzyme *Bst*XI at 55°C, overnight, and the digested products were analysed on 3% agarose gel. To control the digestion reactions, a number of PCR products were randomly selected for sequencing (ABI automated sequencer, model 377, London, UK).

Results

At first all samples were analysed for the Mediterranean mutation and then for the 64 selected samples (9 females

*For correspondence. E-mail: srkhatami@scu.ac.ir.

Keywords. G6PD deficiency; Chatham mutation; PCR-RFLP; *Bst*XI; Khuzestan population.

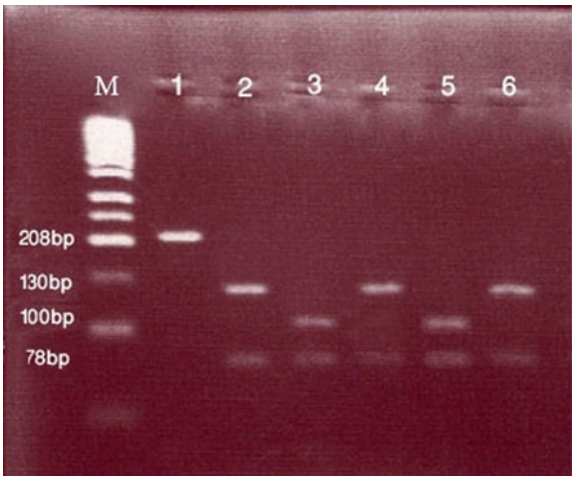

Figure 1. Restriction digestion analysis of PCR products related to G6PD Chatham mutation with *Bst*XI enzyme on 3% agarose gel. From left to right, size marker (50 bp); lane 1, PCR product (208 bp); lanes 3 and 5, the samples have Chatham mutation; the other lanes (2, 4 and 6) have no Chatham mutation.

and 55 males), out of 231 that did not have the Mediterranean mutation were analysed by PCR-RFLP method for the Chatham mutation. After *Bst*XI digestion of the PCR products (a 208-bp fragment of exon 9), the normal samples that had one restriction site showed two fragments (130 and 78 bp) and in mutant samples that had two restriction sites showed three fragments (100, 78 and 30 bp), 100-bp and 30-bp fragments were seen instead of normal 130-bp fragment. In heterozygote samples, we could have 130-bp, 100-bp, 78-bp and 30-bp fragments. As we could not see the 30-bp fragment on the agarose gel only two bands (100 and 78 bp) for hemizygotes (males) and homozygotes (females) and three (130, 100 and 78 bp) for heterozygotes (females) appeared on the gel (figure 1). After which, we also conducted these tests for the remaining samples with the Mediterranean mutation (167 samples) and did not find any evidence of the Chatham mutation in those samples. Thus, the G6PD Chatham genotype was seen in 20 out of 231 patients, including 19 hemizygous males and one homozygous female (20/231 = 8.66%), yielding an allelic frequency of 0.0786 (table 1).

Table 1. Frequency of G6PD Chatham in the Khuzestan Province.

Cases	
Chatham (G1003A)	20
Total G6PDD	231
Chatham frequency (%) in total	8.66
Chatham frequency (%) in males	9.7
Chatham frequency (%) in females	2.8
Allelic frequency (%)	7.86

Discussion

G6PD deficiency is characterized by considerable biochemical and molecular heterogeneity (Mehta *et al.* 2000). The prevalence of G6PD deficiency in Middle East countries (Kuwait, Egypt, Iran, Syria, Lebanon and Jordan) varies greatly, ranging from 1% in Egyptians to 11.5% among Iranians (Usanga and Ameen 2000).

G6PD Chatham has a slightly decreased affinity for glucose-6-phosphate and a significant reduction of *in vitro* thermo stability and can produce neonatal jaundice (Vulliamy *et al.* 1988). Chatham mutation (G1003A) causes class II G6PD deficiency with severe enzymatic deficiency and acute hemolytic anemia, today it is detected in several places through out the world such as Algeria, Philippines (13%) (Hsia *et al.* 1993), Japan, Spain (2%) (Vives Corrons *et al.* 1997), Brazil (0.66%) (Saad *et al.* 1997), Oman (10%) (Daar *et al.* 1996), Indonesia, Kuwait (7.1%) (Samilchuk *et al.* 2003), Jordan (8.82%) (Karadsheh *et al.* 2005), Malaysia, northern and southern Iran (Samilchuk *et al.* 2003) where its frequency has reached 27% (20 out of 74), in north (Mesbah-Namin *et al.* 2002) and 7.3% (5 out of 68) in west and 13.5% (10 out of 74) in south of Iran (Rahimi *et al.* 2006).

Today Chatham mutation is recognized as one of the most common variants world wide (Lwai *et al.* 2001). Highest frequency for G6PD Chatham have been reported from northern Iran (27%) (Mesbah-Namin *et al.* 2002) and lowest frequency reported from Brazil (0.66%) which was carried out in 150 unrelated G6PD deficient individuals (Saad *et al.* 1997).

Khuzestan Province with about 640,000 km^2 area and estimated population of 4,275,000 is located in southwestern Iran and neighbours Iraq to the west. So it would be very important to know more about this population specially the pattern and molecular characterization of diseases like G6PDD which is prevalent in this region. Our results determined that 20 out of 231 patients had Chatham mutation with a frequency of 8.66%. In comparison with other provinces of Iran, the prevalence of Chatham mutation in this study is lower than Mazandaran (27%) (Mesbah-Namin *et al.* 2002), Golestan (26.7% in 71 samples) (Noori-Daloii *et al.* 2004), Khorasan (12%) (Rahimi *et al.* 2006), and Gilan (9.7%) (Rahimi *et al.* 2006), approximately similar to Hormozgan (8.21%) (6 out of 73) (Rahimi *et al.* 2006) but higher than Kermanshah (7.3%) and Sistan and Baluchistan (2.17%) (Rahimi *et al.* 2006).

This result indicates that southwest regions of Iran are similar to the southern region according to the prevalence of Chatham mutation. But prevalence of Chatham mutation in northern regions of Iran is very higher than the south and southwest regions. Also, we can say that the prevalence of this mutation in southwest regions is higher than southeast regions of Iran. The findings maybe due to this fact that there are different ethnic groups and different populations like Kurds in west, Arabs and Farses in southwest and

south, Baluchis in southeast and other populations in northern regions of Iran and also maybe these ethnic groups had obtained this mutation from different sources.

It is also very interesting that our result is approximately similar to the prevalence of Chatham mutation in some western and southern neighbouring Arab countries such as Oman, Kuwait and Jordan. This maybe due to the fact that Arab people live in Khuzestan Province too and maybe these Arab populations which live in these regions have obtained Chatham mutation from a common source. According to these findings, it is not strange if prevalence of Chatham mutation in Khuzestan Province of Iran and the western neighbour country (Iraq) would be very similar as well.

Acknowledgements

Authors thank the personnel of Blood bank center of Ahvaz, specially Mr M. A. Jalalifar and Abuzar and Golestan hospitals of Ahvaz.

References

Beutler E. 1994 G6PD deficiency. *Blood* **84**, 3613–3636.

Beutler E., Blume K. G., Kaplan J. C., Lohr G. W., Ramot B. and Valentine W. N. 1979 International committee for standardization in hematology: recommended screening test for glucose-6-phosphate dehydrogenase (G6PD) deficiency. *Br. J. Haematol.* **43**, 465–467.

Daar S., Vulliamy Y. Y., Kada J. and Mason P. J. 1996 Molecular characterization of G6PD deficiency in Oman. *Hum. Hered.* **46**, 172–176.

Glader B. E. and Lukens J. N. 1999 Hereditary hemolytic anemias associated with abnormalities of erythrocyte glycolysis and nucleotide metabolism. In *Wintrobe's clinical hematology*, 10th edition (ed. G. R. Lee, J. Foerster, J. Lukens, F. Paraskevas, J. P. Greer and G. M. Rodgers) pp. 1160–1175. Williams and Wilkins, Baltimore, USA.

Hsia Y. E., Miyakawa F., Baltazar J., Ching N. S. P., Yuen J., Westwood B. and Beutler E. 1993 Frequency of glucose-6-phosphate dehydrogenase (G6PD) mutations in Chinese, Filipinos, and Laotians from Hawaii. *Hum. Genet.* **92**, 470–476.

Karadsheh N. S., Said L. M., Devaney I. J. and Hoffman E. 2005 Molecular heterogeneity of glucose-6-phosphate dehydrogenase deficiency in Jordan. *Haematologica* **90**, 1693–1694.

Lwai K., Hirono A. and Matsuoka H. 2001 Distribution of glucose-6-phosphate dehydrogenase mutations in southeast Asia. *Hum. Genet.* **108**, 445–449.

Mehta A., Mason P. J. and Vulliamy T. J. 2000 Glucose-6-phosphate dehydrogenase deficiency. *Baillière's Clin. Hematol.* **13**, 21–38.

Mesbah-Namin S. A., Sanati M. H., Mowjoodi A. R., Mason P. J., Vaulliamy T. J. and Noori D. M. R. 2002 Three major G6PD deficient polymorphic variants identified in Mazandaran state of Iran. *Br. J. Haematol.* **117**, 763–764.

Noori-Daloii M. R., Najafi L., Mohammad G. S., Hajebrahimi Z. and Sanati M. H. 2004 Molecular identification of mutations in G6PD gene in patients with favism in Iran. *J. Physiol. Biochem.* **60**, 273–278.

Rahimi Z., Vaisi-Raygani A., Nagel R. L. and Muniz A. 2006 Molecular characterization of glucose-6-phosphate dehydrogenase deficiency in the Kurdish population of Western Iran. *Blood Cells Mol. Dis.* **37**, 91–94.

Saad S. T., Salles T. S., Carvalho M. H. and Costa F. F. 1997 Molecular characterization of glucose-6-phosphate dehydrogenase deficiency in Brazil. *Hum. Hered.* **47**, 17–21.

Samilchuk E., Al-Suliman I., Usanga E. and Al-Awadi S. 2003 Glucose-6-phosphate dehydrogenase (G6PD) mutations and UDP-glucuronosyltransferase promoter polymorphism among G6PD deficient Kuwaitis. *Blood Cells Mol. Dis.* **31**, 201–205.

Usanga E. and Ameen R. 2000 Glucose-6-phosphate dehydrogenase deficiency in Kuwait, Syria, Egypt, Iran, Jordan and Lebanon. *Hum. Hered.* **50**, 158–161.

Vives C. J. L., Zarza R. and Aymerich J. M. 1997 Molecular analysis of glucose-6-dehydrogenase deficiency in Spain. *Sangre (Barc)* **42**, 391–398.

Vulliamy T. J., D'Urso M., Battistuzzi G., Estrada M., Foulkes N. S., Martini G., Calabro V., Poggi V., Giordano R. and Town M. 1988 Diverse point mutations in the human glucose-6-phosphate dehydrogenase gene cause enzyme deficiency and mild or sever hemolytic anemia. *Proc. Natl. Acad. Sci. USA* **85**, 5171–5175.

WHO 1967 Standardization of procedures for the study of glucose-6-phosphate dehydrogenase. *WHO Tech. Rep. Ser.* **366**, 1–53.

Received 16 February 2010, in revised form 1 September 2010; accepted 24 September 2010
Published on the Web: 19 May 2011